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Applications of

RNA Interference

Latest Tools, Applications plus End-User Case Studies
February 10-11, 2003 - Loews Coronado Bay Resort - San Diego, CA

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Since RNAi-based gene silencing was described in worms, research indicates that double-stranded RNA (dsRNA) results in the sequence-specific gene silencing effect that is common to all eukaryotes. These observations are generating enormous interest in the application of RNA interference as a tool to control the expression of specific and multiple genes. Predictions suggest that application of this natural regulatory mechanism will result in a knowledge explosion in cell biology, functional genomics and gene-based therapeutics. The purpose of this conference is to review the utility of RNA interference especially with reference to functional genomics and drug discovery. The aim is to provide a broad overview of emerging methodologies and their industrial utility. The latest advances on RNA interference gene control, knockdown strategies and target validation will be presented.

Scientific Advisory Board
- »**Steve Kay, Ph.D.**, Chief Technology Officer and Senior VicePresident, **Phenomix Corporation**
- »**Eric Lader, Ph.D.**, Business Development, **QIAGEN Sciences**
- »**John Rossi, Ph.D.**, Molecular Biology, **Beckman Research Institute**

Conference Highlights Include:
- »Using siRNA for Target Validation
- »siRNA Delivery in Mammalian Model Systems
- »siRNA Expression Inside Cells: Where?
- »Screening for Therapeutic Cancer Targets Using RNAi
- »Applications in Mammalian Systems
- »siRNA Therapies for HIV Infection

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Agenda for Applications of RNA Interference

February 10-11, 2003 - Loews Coronado Bay Resort - San Diego, CA

Main Conference - Monday, February 10, 2003

| DAY ONE | DAY TWO |

7:30 Registration, Coffee and Breakfast Bakeries

8:50 Chairperson's Welcome and Opening Remarks

Keynote Address

9:00 **Target Validation Requirements in the Pharmaceutical Industry**
Hans Winkler, Ph.D., Global Programme Manager-Target Validation, **AstraZeneca,** United Kingdom
With the increasing availability of the human genome functional analysis of large numbers of genes is becoming more urgent. Medium throughput applications are required to analyze the function of hundreds of genes per year in disease relevant biological effect models. Furthermore, application of similar methods in animal models is required to improve confidence in targets progressed into the drug discovery process. Two major hurdles exist in this endeavor: Firstly, which is the most potent choice of reagent to inhibit target function - possibly indirectly via target abundance and secondly, how can the reagent be delivered into relevant cells at high efficiency and no toxicity both in vivo and in vitro.

9:45 **The Human Dicer Ribonuclease**
David Frendewey, Ph.D., Staff Scientist Biomolecular Sciences, **Regeneron Pharmaceuticals Inc.**
Double-stranded RNA (dsRNA) induces RNA silencing phenomena in a wide spectrum of eukaryotes. The event that triggers the silencing process is the conversion of the dsRNA inducer into small double-stranded interfering RNAs (siRNAs) by the double-strand-specific ribonuclease Dicer. We have purified human Dicer after expression of its cDNA in insect cells and shown that the enzyme is able to convert dsRNA substrates into siRNA-like products. The purified human Dicer also produces an apparently mature let-7 micro RNA (miRNA) by cleavage of a hairpin precursor. Understanding Dicer's different modes of action in the production of siRNAs and miRNAs is an important goal of future research.

10:15 Open Discussion

10:30 Refreshment Break

The Biology of RNA Interference

11:10 **Expressing Small RNAs in Multiple Subcellular Locations**
David Engelke, Ph.D., Professor of Biological Chemistry, **University of Michigan**
Over the last 20 years have attempted to knock down expression of experimental and therapeutic mRNA targets in human cells using

small RNA inhibitors, including antisense, ribozymes, and aptamers. There is currently intense interest in using small, dsRNAs for RNA interference. We have investigated expression of these small RNA types from recombinant DNA cassettes, and have found that the pathway matters.

11:40 Session Discussion

12:00 IBC Sponsored Luncheon in Exhibit Hall

RNAi in Drug Target Discovery

1:20 Chairperson's Welcome and Opening Remarks
Eric Lader, Ph.D., Business Development, **QIAGEN Sciences**

1:30 **Advances in Design, Synthesis, and Transfection of Short Interfering RNA**
Wolfgang Bielke, Ph.D., R&D Scientist, **QIAGEN GmbH**
In mammalian cells, efficient gene silencing requires small interfering RNA (siRNA) complementary to the targeted gene, and a transfection reagent optimized for the delivery of siRNA into cells. QIAGEN's web-based siRNA Design Engine facilitates efficient siRNA design by automating both the design process and a thorough homology analysis of potential siRNA target sequences. High quality, high-throughput siRNA synthesis using QIAGENs TOM chemistry and the current outlook for mid- to high- throughput siRNA transfection into cultured mammalian cells will also be discussed.

2:00 **Efficient Delivery of siRNAs to Mammalian Cells in Culture and In Vivo**
David Lewis, Ph.D., Senior Scientist, **Mirus Corporation**
In order for siRNA to realize its full potential for use in drug target validation studies and as a possible therapeutic, effective siRNA delivery technologies will be required. This paper will describe the application of Mirus Corporation's novel reagents and techniques to enable the delivery of siRNA to a wide variety of cell lines and mammalian model systems.

2:30 **siRNA-Mediated Gene Silencing In Vitro and In Vivo**
Beverly Davidson, Ph.D., Roy J. Carver Professor in Internal Medicine, Professor in Neurology and Physiology & Biophysics, **University of Iowa College of Medicine**
RNA interference is now established as an important biological strategy for gene silencing, but its application to mammalian cells has been limited by nonspecific inhibitory effects of long dsRNA on translation. Here, we describe a viral-mediated delivery mechanism that results in specific silencing of targeted genes through expression of small interfering RNA (siRNA). We establish proof of principle by markedly diminishing expression of exogenous and endogenous genes in vitro and in vivo in brain and liver, and further apply this strategy to a model system of a major class of neurodegenerative disorders, the polyglutamine diseases, to show reduced polyglutamine aggregation in cells. This viral-mediated strategy should prove generally useful in reducing expression of target genes to model biological processes or to provide therapy for dominant human diseases.

3:00 Refreshment Break in Exhibit Hall

3:45 **RNAi and Antisense Technologies: Applications for Drug Target Discovery and Validation**
Dmitry Samarsky, Ph.D. Technology Development Manager, **Sequitur, Inc.**
RNAi and antisense technologies enable systematic gene function analysis and drug target validation. We will present data that demonstrate and compare the intracellular delivery, specificity and activity of siRNAs and antisense cleavers and blockers. We will give examples of gene knock-downs with siRNA and antisense compounds, as well as phenotypic results in a variety of disease model systems, including the validation of the Alzheimer's beta-secretase. The data obtained with siRNA will also include: role of nucleotide modifications for siRNA activity, improved methods for monitoring of siRNA intracellular delivery, and increasing siRNA efficiency and specificity. We will close with a discussion of our OmniScreen program, which combines siRNA and antisense compounds with high throughput phenotypic assays, and allows for one-step target discovery and validation.

4:15 **Reversing Trends in Mouse Forward Genetics to Create Novel Target Space**
Steve Kay, Ph.D., Chief Technology Officer and Senior Vice President, **Phenomix Corporation**
Rapid advances in genomics technologies have suggested a wealth of new therapeutic targets, but typically these targets are weakly validated with only circumstantial evidence linking them to human disease. The next challenge is testing gene-to-disease connections in a relevant animal model, a time-consuming and uncertain process using conventional reverse genetic approaches such as knockout and transgenic mice. In contrast, forward genetics proceeds by measuring a physiological process with a high degree of disease relevance, then identifying the gene products that impinge on this process. This 'phenotype-first' approach solves the bottleneck of target validation by using clinically relevant assays in a whole animal mammalian system as a discovery platform. As an unbiased approach to gene discovery and validation, forward genetics will open novel drug target space and increase the success rate of drug development. We will discuss how this approach can be combined with acute validation methodologies such as RNAi in intact animals.

4:45 Session Discussion

5:00-6:30 Networking Cocktail Reception in Exhibit Hall

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Main Conference -Tuesday, February 11, 2003

| DAY ONE | DAY TWO |

7:30 Coffee and Breakfast Bakeries

Case Study RNAi Applications

8:20 Chairperson's Welcome and Opening Remarks

8:30 **Genome-Wide Knockdown Strategies - RNAi Applications**
Greg Plowman, M.D., Ph.D., Vice President, Pharmaceutical Research, **Exelixis Pharmaceuticals, Inc.**
Strategies such as RNA, combined with access to the complete genome sequence of humans and several model organisms, make it possible to systematically study gene function. RNAi was first described in worms and has since been adapted for use in other invertebrates, cell lines, and mammals. The versatility of RNAi has resulted in its rapid assimilation into the drug discovery process. The talk will review our work on worm, fly, S2 cell and mammalian cell based approaches.

9:00 **TBA**

9:30 **Utilizing RNAi in the Functional Validation of Putative Therapeutic Cancer Targets**
Qi Wang, Ph.D., Scientist, **EOS Biotechnology Inc.**
Using proprietary DNA microarrays, a set of cancer-selective genes has been identified. RNAi is utilized in cancer cell lines to reduce target protein expression and thus assess the functional import of these genes in tumor growth. Data will be presented for known and novel targets illustrating the effectiveness of RNAi in allowing for accelerated functional validation of anti-cancer targets.

10:00 Refreshment Break in Exhibit Hall

10:45 **Incorporating siRNA into Inverse Genomics™ for Target Discovery and Validation**
Flossie Wong-Staal, Ph.D., Chief Scientific Officer, **Immusol, Inc.**
We have developed a technology platform called Inverse Genomics to identify and functionally validate therapeutic drug target genes in different disease areas. From the ribozyme's substrate binding sequence, a biologically relevant target gene, which is linked to the therapeutic effect, can be identified. Using this technology, we have identified functional genes involved in regulation of tumor suppressor genes, HCV and HIV infection and neuronal cell apoptosis. Recently, we have used siRNA as an alternate or adjunctive tool for the validation phase of Inverse Genomics. Furthermore, we are developing randomized siRNA vector libraries for the discovery phase of Inverse Genomics.

11:15 **Combining RNA Interference with Mouse Knockout Technologies in Drug Discovery Research**
Christian W. Siebel, Ph.D., Associate Director of Molecular Biology, **Deltagen Inc.**
Deltagen provides key functional information from making and analyzing mouse knockout mutations in thousands of drug target genes. To complement this high-throughput knockout approach, we are developing gene expression "knockdown" technologies, including RNA interference and ribozymes. We have exploited RNAi in mammalian cell assays to illuminate biochemical mechanisms of drug target function and to improve gene targeting techniques. We are currently trying to advance RNAi expression and delivery technologies to achieve our long term goal of regulating gene expression in adult animals.

11:45 Session Discussion

12:00 Lunch on your own

1:30 **Integration of RNAi in the Drug Discovery Pipeline**
Eric Von Hofe, Ph.D., Program Director, **Millennium Pharmaceuticals, Inc.**
The ability to modulate target gene expression in a reproducible, robust manner has a number of potential uses during target validation and drug discovery. In addition to its use as a guide in developing 'clean' small molecule inhibitors of disease relevant targets, the potential for using RNAi in high-throughput cell-based assays (e.g., pathway analysis) offers significant advantages. Millennium's efforts in both these areas will be discussed.

2:00 **Various RNAi Methodologies in Functional Genomics**
Seth Crosby, M.D., Scientist, **Pharmacia Corp.**
(Abstract unavailable at time of posting.)

2:30 Refreshment Break in Exhibit Hall

RNAi as a Therapeutic Strategy

3:00 Chairperson's Opening Remarks

3:10 **Ribozyme, RNA Decoy and siRNA Therapies for HIV Infection.**
John Rossi, Ph.D., Professor, Department of Molecular Biology, **Beckman Research Institute**
Our laboratory has been developing novel RNA based therapeutics for the treatment of HIV infection and other diseases, including cancers. Our basic strategy is to deliver genes capable of expressing these RNAs via lentiviral and retroviral vectors to the appropriate target cells. For HIV targeting we are utilizing hematopoietic stem cells which when infused into patients can result in long term production of the antiviral RNAs in all the hematopoietic lineages that are infectible by HIV. The strategies that are being developed for expression of these RNAs are applicable to a variety of targets, including oncogenes and mutant cellular transcripts. Examples for therapeutic siRNAs and ribozymes will also be presented for the fusion protein encoding Ewing's sarcoma oncogene as well as expanded triplet repeat containing transcripts characteristic of myotonic dystrophy.

3:40 **Tumor Inhibition by RNAi-Mediated Anti-angiogenesis in Xenograft Models**
Martin Woodle, Ph.D., President & CEO, **Intradigm Corporation**
Intradigm has developed a propriety system for high-efficiency delivery in the xenograft tumor model, of various forms of RNAi. This delivery system enables the RNAi-mediated down regulation of endogenous genes, hVEGF165 and mVEGFR2, that play key roles in angiogenesis pathway. This paper will describe how this technology is being used to validate novel cancer targets ready for pre-clinical development of cancer therapeutics.

4:10 **Title unavailable at time of print**
Christophe Echeverri, Ph.D., CEO, **Cenix BioScience,** Germany
(Abstract unavailable at time of print. Please check our website, www.LifeSciencesInfo.com/RNAi for program updates.)

[redacted]

4:40 Close of Conference

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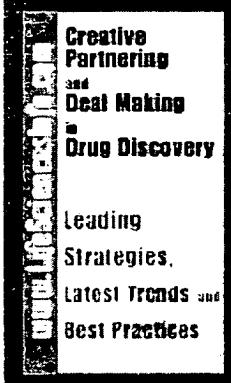

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February 10-11, 2003 - Loews Coronado Bay Resort - San Diego, CA

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